

WHAT IS CLAIMED IS:

1 1. A nucleic acid encoding a Diphtheria toxin fusion protein comprising
2 (1) residues 1-388 of Diphtheria toxin, wherein the native furin cleavage site
3 has been substituted for a cleavage site for a matrix metalloproteinase or a plasminogen
4 activator; and
5 (2) a heterologous polypeptide, wherein the heterologous polypeptide
6 specifically binds to a protein overexpressed on the surface of a cell.

1 2. The nucleic acid of claim 1, wherein the matrix metalloproteinase is
2 selected from the group consisting of MMP-2 (gelatinase A), MMP-9 (gelatinase B) and
3 membrane-type1 MMP (MT1-MMP).

1 3. The nucleic acid of claim 1, wherein the plasminogen activator is
2 selected from the group consisting of tissue plasminogen activator (t-PA) and urokinase
3 plasminogen activator (u-PA).

1 4. The nucleic acid of claim 1, wherein the matrix metalloproteinase
2 cleavage sites are GPLGMLSQ and GPLGLWAQ.

1 5. The nucleic acid of claim 1, wherein the plasminogen activator
2 cleavage site is selected from the group consisting of QRGRSA, GSGRSA and GSGKSA.

1 6. The nucleic acid of claim 1, wherein the protein overexpressed on the
2 surface of a cell is a receptor.

1 7. The nucleic acid of claim 1, wherein the heterologous polypeptide
2 comprises a cytokine.

1 8. The nucleic acid of claim 1, wherein the heterologous polypeptide
2 comprises a growth factor.

1 9. The nucleic acid of claim 1, wherein the heterologous polypeptide is a
2 member selected from the group consisting of: IL-2, GM-CSF, and EGF.

1 10. The nucleic acid of claim 1, comprising the nucleotide sequence set
2 forth in SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13.

- 1 11. A vector comprising the nucleic acid of claim 1.
- 1 12. The nucleic acid of claim 6, wherein the cell is a cancer cell.
- 1 13. The nucleic acid of claim 7, wherein the heterologous polypeptide
2 comprises GM-CSF.
- 1 14. The nucleic acid of claim 7, wherein the heterologous polypeptide
2 comprises IL-2.
- 1 15. The nucleic acid of claim 8, wherein the heterologous polypeptide
2 comprises EGF.
- 1 16. A nucleic acid encoding a Diphtheria toxin fusion protein comprising
2 (1) residues 1-388 of Diphtheria toxin, wherein the native furin cleavage site
3 has been substituted for a cleavage site for a urokinase a plasminogen activator; and
4 (2) GM-CSF.
- 1 17. A polypeptide encoded by the nucleic acid of claim 1.
- 1 18. A polypeptide encoded by the nucleic acid of claim 10.
- 1 19. A polypeptide encoded by the nucleic acid of claim 16
- 1 20. A host cell comprising the vector of claim 11.
- 1 21. The nucleic acid of claim 12, wherein the cancer is leukemia.
- 1 22. The nucleic acid of claim 12, wherein the cancer is acute myelogenous
2 leukemia.
- 1 23. A pharmaceutical composition comprising the protein of claim 18 and
2 a pharmaceutically acceptable carrier.
- 1 24. A method of treating cancer, the method comprising administering to a
2 subject a Diphtheria toxin fusion protein comprising

3 (1) residues 1-388 of Diphtheria toxin, wherein the native furin cleavage site
4 has been substituted for a cleavage site for a matrix metalloproteinase or a plasminogen
5 activator; and

6 (2) a heterologous polypeptide, wherein the heterologous polypeptide
7 specifically binds to a protein overexpressed on the surface of a cell.

1 25. The method of claim 24, wherein the matrix metalloproteinase is
2 selected from the group consisting of MMP-2 (gelatinase A), MMP-9 (gelatinase B) and
3 membrane-type1 MMP (MT1-MMP).

1 26. The method of claim 24, wherein the plasminogen activator is selected
2 from the group consisting of t-PA and u-PA.

1 27. The method of claim 24, wherein the matrix metalloproteinase
2 cleavage sites are GPLGMLSQ and GPLGLWAQ.

1 28. The method of claim 24, wherein the plasminogen activator cleavage
2 site is selected from the group consisting of QRGRSA, GSGRSA and GSGKSA.

1 29. The method of claim 24, wherein the protein overexpressed on the
2 surface of a cell is a receptor.

1 30. The method of claim 24, wherein the cell is a cancer cell.

1 31. The method of claim 24, wherein the heterologous polypeptide
2 comprises a cytokine.

1 32. The method of claim 24, wherein the heterologous polypeptide
2 comprises a growth factor.

1 33. The method of claim 24, wherein the fusion protein is encoded by the
2 nucleotide sequence set forth in SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13.

1 34. The method of claim 30, wherein the cancer is leukemia.

1 35. The method of claim 30, wherein the cancer is acute myelogenous
2 leukemia.

1 36. The method of claim 31, wherein the heterologous polypeptide
2 comprises GM-CSF.

1 37. The method of claim 31, wherein the heterologous polypeptide
2 comprises IL-2.

1 38. The method of claim 32, wherein the heterologous polypeptide
2 comprises EGF.

1 39. The method of claim 24, wherein the Diphtheria toxin fusion protein
2 comprises:

3 (1) residues 1-388 of Diphtheria toxin, wherein the native furin cleavage site
4 has been substituted for a cleavage site for a urokinase plasminogen activator; and
5 (2) GM-CSF.

1 40. A method of targeting a compound to a cell overexpressing a cytokine
2 receptor or a growth factor receptor, the method comprising the steps of:

3 administering to the cell Diphtheria toxin fusion protein comprising
4 (1) residues 1-388 of Diphtheria toxin, wherein the native furin cleavage site
5 has been substituted for a cleavage site for a matrix metalloproteinase or a plasminogen
6 activator and wherein the Diphtheria toxin is cleaved by a matrix metalloproteinase or a
7 plasminogen activator; and

8 (2) a heterologous polypeptide, wherein the heterologous polypeptide
9 specifically binds to a cytokine receptor or a growth factor receptor.

1 41. The method of claim 40, wherein the cell also overexpresses a matrix
2 metalloproteinase, a tissue plasminogen activator, or a urokinase plasminogen activator.

1 42. The method of claim 40, wherein the matrix metalloproteinase is
2 selected from the group consisting of MMP-2 (gelatinase A), MMP-9 (gelatinase B) and
3 membrane-type1 MMP (MT1-MMP).

1 43. The method of claim 40, wherein the plasminogen activator is selected
2 from the group consisting of t-PA and u-PA.

1 44. The method of claim 40, wherein the matrix metalloproteinase
2 cleavage sites are GPLGMLSQ and GPLGLWAQ.

1 45. The method of claim 40, wherein the plasminogen activator cleavage
2 site is selected from the group consisting of QRGRSA, GSGRSA and GSGKSA.

1 46. The method of claim 40, wherein the cancer cell is a leukemia cell.

1 47. The method of claim 40, wherein the cancer cell is an acute
2 myelogenous leukemia cell.

1 48. The method of claim 40, wherein the Diphtheria toxin fusion protein
2 comprises

3 (1) residues 1-388 of Diphtheria toxin, wherein the native furin cleavage site
4 has been substituted for a cleavage site for a urokinase plasminogen activator; and
5 (2) GM-CSF.

1 49. An isolated nucleic acid comprising the sequence set forth in any one
2 of SEQ ID NOS: 2-18.